

AMENDMENT UNDER 37 C.F.R. § 1.121(C)

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Claim 1 (Previously Presented) A method of producing a heterologous peptide, polypeptide or protein in a lactic acid bacterium, the method comprising the steps of

- (i) constructing a recombinant lactic acid bacterium comprising a nucleotide sequence coding for the heterologous peptide, polypeptide or protein and operably linked thereto, appropriate regulatory nucleotide sequences to control the expression of the coding sequence,
 - (ii) cultivating said recombinant bacterium under fed-batch or continuous cultivation conditions in a chemically defined medium, to express the nucleotide sequence, and
 - (iii) harvesting the recombinant bacterium or the peptide, polypeptide or protein,
- wherein the concentration of glucose is kept at a pre-selected concentration of at least about 0.5 g/L by controlled feeding of glucose.

Claim 2 (Previously Presented) A method according to claim 1 wherein the recombinant bacterium comprises a constitutive promoter operably linked to the coding sequence.

Claim 3 (Previously Presented) A method according to claim 1 wherein the recombinant bacterium comprises a regulatable promoter operably linked to the coding sequence.

Claim 4 (Previously Presented) A method according to claim 3 wherein the regulatable promoter is regulated by accumulation of a metabolite intracellularly or in the medium.

Claim 5 (Previously Presented) A method according to claim 3 wherein the regulatable promoter is derived from a lactic acid bacterium.

Claim 6 (Previously Presented) A method according to claim 5 wherein the regulatable promoter is the P170 promoter disclosed in WO 98/10079 or a derivative thereof.

Claim 7 (Previously Presented) A method according to claim 3 wherein the promoter is introduced into the lactic acid bacterium on an autonomously replicating replicon.

Claim 8 (Previously Presented) A method according to claim 3 wherein the promoter is a promoter not naturally associated with the nucleotide sequence coding for the heterologous peptide, polypeptide or protein.

Claim 9 (Original) A method according to claim 1 wherein the heterologous peptide, polypeptide or protein is selected from the group consisting of an enzyme and a pharmaceutically active compound.

Claim 10 (Original) A method according to claim 1 wherein the coding nucleotide sequence is operably linked to a nucleotide sequence coding for a signal peptide (SP).

Claim 11 (Currently Amended) A method according to claim 10 wherein the signal peptide is selected from the group consisting of the ~~usp45~~ Usp45 signal peptide and the signal peptide having the sequence MKFNKKRVAIATFIALIFVSFFTSSQDAQAAERS (SEQ ID NO: 1).

Claims 12-13 (Cancelled)

Claim 14 (Previously Presented) A method according to claim 1 wherein the control of feeding of glucose to the medium is linked to pH control.

Claims 15-16 (Cancelled)

Claim 17 (Previously Presented) A method according to claim 1 wherein the yield of heterologous peptide, polypeptide or protein is at least 5 mg/L.

Claims 18-23 (Cancelled)

Claim 24 (Previously Presented) A method according to claim 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8

L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. **119**:736-747;

^b Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

^c Micronutrients: 0.003 μM (NH₄)₆(MoO₇)₂₄, 0.4 μM H₃BO₄, 0.03 μM CoCl₂, 0.01 μM CuSO₄, 0.08 μM MnCl₂ and 0.01 μM ZnSO₄, or

wherein the components of said chemically defined medium are present in three-fold or five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

Claims 25-26 (Cancelled)

Claim 27 (Previously Presented) A method according to claim 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1

L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. **119**:736-747;

^b Vitamins: 0.4 μ M biotin, 10 μ M pyridoxal-HCl, 2.3 μ M folic acid, 2.6 μ M riboflavin, 8 μ M niacinamide, 3 μ M thiamine-HCl and 2 μ M pantothenate;

^c Micronutrients: 0.003 μ M (NH₄)₆(MoO₇)₂₄, 0.4 μ M H₃BO₄, 0.03 μ M CoCl₂, 0.01 μ M CuSO₄, 0.08 μ M MnCl₂ and 0.01 μ M ZnSO₄;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L, or

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L and wherein the components of said chemically defined medium are present in three-fold or five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

Claims 28-29 (Cancelled)

Claim 30 (Previously Presented) A method of producing a heterologous peptide, polypeptide or protein in a lactic acid bacterium, the method comprising the steps of

- (i) constructing a recombinant lactic acid bacterium comprising a nucleotide sequence coding for the heterologous peptide, polypeptide or protein and operably linked thereto, appropriate regulatory nucleotide sequences to control the expression of the coding sequence,
- (ii) cultivating said recombinant bacterium under fed-batch or continuous cultivation conditions in a chemically defined medium supplemented with yeast extract, to express the nucleotide sequence, and
- (iii) harvesting the recombinant bacterium or the peptide, polypeptide or protein,

wherein the concentration of glucose is kept at a pre-selected concentration of at least about 0.5 g/L by controlled feeding of glucose.

Claim 31 (Previously Presented) A method according to claim 30 wherein the recombinant bacterium comprises a constitutive promoter operably linked to the coding sequence.

Claim 32 (Previously Presented) A method according to claim 30 wherein the recombinant bacterium comprises a regulatable promoter operably linked to the coding sequence.

Claim 33 (Previously Presented) A method according to claim 32 wherein the regulatable promoter is regulated by accumulation of a metabolite intracellularly or in the medium.

Claim 34 (Previously Presented) A method according to claim 32 wherein the regulatable promoter is derived from a lactic acid bacterium.

Claim 35 (Previously Presented) A method according to claim 34 wherein the regulatable promoter is the P170 promoter disclosed in WO 98/10079 or a derivative thereof.

Claim 36 (Previously Presented) A method according to claim 32 wherein the promoter is introduced into the lactic acid bacterium on an autonomously replicating replicon.

Claim 37 (Previously Presented) A method according to claim 32 wherein the promoter is a promoter not naturally associated with the nucleotide sequence coding for the heterologous peptide, polypeptide or protein.

Claim 38 (Previously Presented) A method according to claim 30 wherein the heterologous peptide, polypeptide or protein is selected from the group consisting of an enzyme and a pharmaceutically active compound.

Claim 39 (Previously Presented) A method according to claim 30 wherein the coding nucleotide sequence is operably linked to a nucleotide sequence coding for a signal peptide (SP).

Claim 40 (Previously Presented) A method according to claim 39 wherein the signal peptide is selected from the group consisting of the ~~usp45~~ Usp45 signal peptide and the signal peptide having the sequence MKFNKKRVAIATFIALIFVSFFTISSQDAQAAERS (SEQ ID NO: 1).

Claim 41 (Previously Presented) A method according to claim 30 wherein the control of feeding of glucose to the medium is linked to pH control.

Claim 42 (Previously Presented) A method according to claim 30 wherein the amount of yeast extract is in the range of 0.1-10 g/L.

Claim 43 (Previously Presented) A method according to claim 30 wherein the yield of heterologous peptide, polypeptide or protein is at least 5 mg/L.

Claim 44 (Previously Presented) A method according to claim 30 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4

L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. **119**:736-747;

^b Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

^c Micronutrients: 0.003 μM (NH₄)₆(MoO₇)₂₄, 0.4 μM H₃BO₄, 0.03 μM CoCl₂, 0.01 μM CuSO₄, 0.08 μM MnCl₂ and 0.01 μM ZnSO₄;

wherein the components of said chemically defined medium are present in three-fold or five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

Claim 45 (Previously Presented) A method according to claim 30 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8

L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. **119**:736-747;

^b Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

^c Micronutrients: 0.003 μM (NH₄)₆(MoO₇)₂₄, 0.4 μM H₃BO₄, 0.03 μM CoCl₂, 0.01 μM CuSO₄, 0.08 μM MnCl₂ and 0.01 μM ZnSO₄;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L, or

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L and wherein the components of said chemically defined medium are present in three-fold or five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.